

APHL All Member Call: Highly Pathogenic Avian Influenza A(H5N1)

August 21, 2024

Call Summary

Contact for Questions or Suggestions

APHL Influenza Contact: fluquestions@aphl.org

Updates from the Field: Review of FDA's Influenza A(H5N1) Milk Testing Protocol (*Angela Gail Swinford and Jaqueline Woods, FDA*)

FDA has shared their [Standard Operating Procedure for Subtyping of HPAI H5 in Milk and Milk Products Using RT-qPCR](#) through the [Bacteriological Analytical Manual](#) (BAM) Additional Chemistry and Microbiology Resources Used by the Foods Program. The method is suitable for fluid milk, sour cream, butter and cheeses. Note that Table 6 has updated DNase/RNase-free water volumes for the buffer mix (1760 µl, 1610 µl). FDA is using synthetic G block probes as positive controls.

Updates from the Field: Colorado Situational Update (*Allison Kohnen and Shannon Matzinger, Colorado Department of Health and Environment*)

Over the last several months, Colorado has had 64 infected dairies diagnosed prompting the State Veterinarian's office to implement a mandatory bulk tank surveillance program. CDHE has been on site at dairies, working directly with producers and dairy workers to monitor for illness. Several anecdotal reports of conjunctivitis in workers over the last few months. The first detection of H5 in a dairy worker occurred in mid-July involving one of the seven workers that were testing in that facility. This was followed by new detections in poultry, and a strong, robust epidemiology response to that situation.

Coordination for the field programs included coordination with field veterinarians, field epidemiologists, public health nurses, and bilingual staff to implement the surveillance in individuals and the field. Most human samples were collected on farms, including conjunctival swabs and nasopharyngeal (NP) swabs. Prophylactic Tamiflu was administered for individuals with pending results. The poultry field response was initiated from reports of symptomatic workers by on site case managers. This work is being documented and will be featured in an upcoming [Morbidity and Mortality Weekly Report \(MMWR\)](#) article.

The first round of testing yielded some positive detections and inconclusive results, with very low viral loads and only had one target positive (either the H5a or universal influenza A). These samples were immediately sent to CDC and retested in Colorado. Paired NP and conjunctival samples were used for verification of a new specimen type. The laboratory performed QIAGEN manual extraction for H5 subtyping, as well as the QIAGEN EZ1 (after inactivation). Several symptomatic cases were identified as COVID-19 by running the influenza A/B and SARS-CoV-2 assay in parallel to the other testing.

Colorado Milk Testing Mandate (*Kristy Pabilonia, CSU*)

The Veterinary Diagnostic Laboratories at Colorado State University is part of the National Animal Health Laboratory Network (NAHLN). On July 22, 2024 Colorado issued a [testing order](#) mandating dairy cow farms in the state submit weekly bulk-tank samples for testing. This order has standardized the types of samples, information and submitters for milk testing in the state. Reports are sent back to the regulatory authorities, producers and their veterinarian, with All results transmitted to NAHLN via HL7 messaging t.

The primary challenges have been primarily related to the sample type and data management. The milk samples are not physically uniform in matrix, leading to some sample inhibition in PCR. Typically, the samples have very low Ct values, requiring all samples to be diluted. The samples may be submitted with incomplete or incorrect information for required data fields, necessitating extensive follow up.

Updates from the Field: Establishing Milk Testing in Massachusetts (*Sanjib Bhattacharyya, MA DPH*)

The Massachusetts Public Health Laboratory is currently establishing protocols for milk testing for H5. They reviewed and modified the risk assessment for HPAI and whole milk testing from Wadsworth Center, New York State. The laboratory spiked pasteurized whole milk samples with H1 and H3 to ensure the extraction and PCR were working well. The Kingfisher flex was used for extraction and the CDC influenza assay was used to detect the virus. The next step will be working towards spiking raw milk with H1, H3 and then, including the synthetic H5 control and the FDA primers and probes. MA plans to optimize the protocol soon for HPAI H5 subtyping in milk and milk products. The laboratory is also in touch with one of their Pathogen Genomics Centers of Excellence (PGCoE) partners, the Broad Institute, to explore additional method development.

CDC Laboratory Testing Updates (*Todd Davis, CDC*)

The CDC FluSC2 Multiplex has been recently updated and the Instructions for Use (IFU) now include universal transport media (UTM). PHLs should receive communications from the International Reagent Resource (IRR) with this updated information.

The new lot of the H5b primer probe component in the H5 assay has been completed and is now available in the IRR inventory. Laboratories should continue to use the existing kits that were previously distributed without H5b component until those kits are exhausted before ordering new kits that have both H5a and H5b components.

CDC is starting some preliminary communications with FDA to have conjunctival specimens added as an approved sample type to our 510(k) assays, with the goal of avoiding further extensions of enforcement discretion. This may take some time, so there may be one another extension when this current enforcement discretion ends in November.

Influenza A(H1/H3)v Update (*Todd Davis, CDC*)

CDC encourages PHLs to remain vigilant for atypical results with influenza typing and subtyping algorithms, especially during the agricultural fair season. This year there have been four confirmed cases of influenza A(H1N2) variant virus following exposure to swine (three from PA, one from OH). Most of the cases were mild, though the case from Ohio was considered severe illness, likely due to underlying medical conditions. CDC wants to identify novel cases, but it also is a source of specimens that help in pandemic preparedness posturing.

CDC compares these viruses to circulating strains in animal hosts and works to isolate them for antiviral susceptibility testing. This ensures that antiviral drugs are still effective against these variant strains. The CDC also performs antigenic characterization against either wild type viruses or reassortment strains recommended

as candidate vaccine viruses. In some cases, sequence analysis is also necessary to identify the specific neuraminidase that is associated with these variant cases.

There have also been three cases in 2024 of influenza A(H3N2) variant viruses (one each from Saskatchewan, Colorado, and Michigan). The Colorado case involved exposure to pigs after attending an agricultural fair. The virus hemagglutinin lineage is derived from a recent human seasonal H3 virus that was introduced to the US swine population in the early 2010s. This group of viruses provides some immune protection for adults who have been exposed to that variety of seasonal H3 viruses. However, children that were not exposed and haven't been vaccinated with antigenically related strains have less immunity to these strains. This strain could potentially reemerge in the human population and the CDC monitors for onward transmission from person to person. There is a library of H3 variant candidate vaccine strains that are produced every couple of years to keep pace with the evolutionary and antigenic changes that are detected in these H3 variant viruses.

Questions and Answers (Q&A)

Q: Is milk testing done under BSL-3 conditions?

A: Diagnostic samples may be performed until BSL-2, but CSU is using BSL-3 space with the appropriate PPE as a best practice. FDA labs analyze raw milk products, samples for HPAI and BSL-2 plus lab space with BSL-3 practices. APHL has shared a Risk Assessment [\[add link\]](#).

Q: Are there any plans to validate other specimen collection preservatives such as eNAT from COPAN?

A: Not at this time.

Q: Are there any updates on extraction instrumentation additions for CDC's 510(k) cleared influenza assays or the EUA?

A: Not at this time.

Next APHL HPAI Call: September 4 at 2:00pm ET

Next APHL ID Issues Call: August 28 at 3:00pm ET